Developmental Biology **206,** 157–162 (1999)
Article ID dbio.1998.9143, available online at http://www.idealibrary.com on IDEAL®

# of Spemann's Organizer Is Independent of Wnt Signaling

Micheline N. Laurent and Ken W. Y. Cho[1]

Department of Developmental and Cell Biology and the Developmental Biology Center, University of California at Irvine, Irvine, California 92697-2300

The *Xenopus* homeobox gene *twin* is involved in the Wnt-mediated induction of Spemann's organizer. Additionally, several lines of evidence indicate that bone morphogenetic proteins (BMPs) play a role in repressing the formation of the organizer by antagonizing the expression of genes involved in organizer establishment. In order to determine at what level BMPs exert their effect, we measured the activity of different genes expressed within the organizer region. We report that BMP signaling can antagonize the induction of the dorsal-specific gene *goosecoid* but is unable to affect Wnt signaling at the level of *twin*. These results suggest that the antagonistic activities of BMPs in organizer formation occur postzygotically, independent of *twin* regulation, and that Wnt-like dorsal determinant signaling pathways do not crosstalk with BMPs. © 1999 Academic Press

INTRODUCTION

Establishment of the dorsoventral body axis in *Xenopus* requires the activity of Spemann's organizer, an embryonic signaling center essential for the induction of dorsal structures. The events leading to organizer formation are initiated at fertilization when sperm entry triggers the rotation of the outer egg cortex relative to the inner endoplasm, with the point of sperm entry predicting the ventral side of the embryo (Gerhart *et al.*, 1989). This cortical rotation results in the distribution of vegetally localized dorsal determinants to the dorsal side of the embryo, the location of the prospective organizer (reviewed in Gerhart *et al.*, 1989). Exposure of embryos to ultraviolet (UV) light prior to the first cleavage results in hyperventralized embryos lacking dorsal structures (Scharf and Gerhart, 1983) since this treatment blocks organizer establishment by preventing the movement of the dorsal determinants to the dorsal side of the embryo (Holowacz and Elinson, 1993; Kageura, 1997). The dorsal overexpression of bone morphogenetic proteins (BMPs) produces a similar phenotype and results in the repression of organizer-specific genes such as *goosecoid* (*Xgsc*) (Dale *et al.*, 1992; Jones *et al.*, 1992; Hawley *et al.*, 1995; Jones *et al.*, 1996). However, whether BMPs interfere with dorsal determinant activity, or by an alternative mechanism, is not well understood.

Recently, two *Xenopus* Wnt target genes, *Xsiamois* (*Xsia*) and *Xtwin* (*Xtwn*), were identified based on their ability to induce ectopic organizer tissue when overexpressed in embryos (Lemaire *et al.*, 1995; Laurent *et al.*, 1997). Several lines of evidence indicate that *Xsia* and *Xtwn* are regulated by Wnt-like dorsal determinants. First, spatiotemporal expression of these genes is consistent with dorsal determinant activity as both *Xsia* and *Xtwn* are detected within the dorsal marginal zone at MBT (Lemaire *et al.*, 1995, Laurent *et al.*, 1997). Second, both *Xsia* and *Xtwn* are specifically induced in response to ectopic Wnt-type signals via consensus LEF/Tcf3 binding sites present in the *Xsia* and *Xtwn* promoters (Brannon *et al.*, 1997; Laurent *et al.*, 1997). LEF/Tcf are transcriptional factors which physically associate with β-catenin (Behrens *et al.*, 1996; Huber *et al.*, 1996; Molenaar *et al.*, 1996) to regulate downstream Wnt target genes. These LEF binding sites are required *in vivo* since mutations within these sites abrogate normal expression and prevent *Xsia* and *Xtwn* reporter genes from responding to Wnt signals (Brannon *et al.*, 1997, Laurent *et al.*, 1997). These results suggest that *Xtwn* and *Xsia* are direct target genes regulated by Wnt-type dorsal determinants. While *Xtwn* expression is primarily induced in response to Wnt-mediated signaling, Smad-mediated ac-

[1] To whom correspondence should be addressed. Fax: (949) 824-4067 or 4709. E-mail: kwcho@uci.edu.

0012-1606/99 $30.00

tivin signaling has also been implicated in *Xsia* induction (Crease *et al.*, 1998).

Organizer specification appears to require input from both the Wnt-like dorsal determinants and the members of the TGF-β superfamily. Support for this interaction has been provided by analysis of the regulation of the organizer-specific homeobox gene, *Xgsc*. Molecular studies demonstrate that *Xgsc* expression is mediated by the combined effects of two regions of the promoter, the distal element (DE) and the proximal element (PE). The DE responds directly to dorsal mesoderm-inducing signals such as those mimicked by activin and BVg1 (members of the TGF-β superfamily), whereas the PE responds indirectly to Wnt signaling following the induction of the *Xtwn/Xsia*-type homeobox genes (Watabe *et al.*, 1995; Fan and Sokol, 1997; Kessler, 1997; Laurent *et al.*, 1997).

While organizer activity is positively regulated by TGF-β molecules such as activin and Vg1, overexpression of the BMPs, a subfamily of TGF-β molecules, antagonizes organizer activity and is important in the specification of ventral cell fates. Using reporter genes containing various growth factor-responsive elements of *Xgsc*, BMP signaling has been demonstrated to attenuate activin/BVg1-type induction of *Xgsc* expression by competing for a limited pool of Smad4, a common signaling component shared by both of these pathways (Candia *et al.*, 1997). As overexpression of BMPs dorsally results in the abrogation of *Xgsc* expression and a hyperventralized phenotype indistinguishable from those of UV-irradiated embryos in which cortical rotation is blocked, we wished to determine whether the mechanisms dictating ventralization are similar. In this article we show that BMP ventralization occurs by a distinct process. While BMP signaling can interfere with the expression of the organizer marker gene *Xgsc*, it is unable to alter the Wnt-mediated induction of *Xtwn* and *Xsia*. These results suggest that BMP antagonism of the organizer occurs independent of the dorsal determinants in their initiation of organizer function.

MATERIALS AND METHODS

Embryo Manipulations and RNA Injections

Eggs were fertilized *in vitro* and dejellied and resultant embryos cultivated as described previously (Cho *et al.*, 1991). Staging was according to Nieuwkoop and Faber (1967). Dorsoventral polarity was determined and embryos were selected based upon appropriate pigment distributions. The dorsal regions of embryos were marked with Nile blue dye for orientation during explant dissections. For CABR and DNBR RNA injections, RNA was prepared as described in Candia *et al.* (1997).

RT-PCR Analysis

RT-PCR analysis was performed as described previously (Blitz and Cho, 1995). An exception was made in Fig. 1, in which 22 cycles of PCR were performed for the *histone H4* primers. The *Xtwn* and *Xsia* primers are described in Laurent *et al.* (1997). The *Xgsc* primers used were described in Watabe *et al.* (1995). The *histone H4* primers were described in Blitz and Cho (1995). Xvent primers were described in Candia *et al.* (1997). Chordin primers were AACTGCCAGGACTGGATGGT and GGCAGGATTTAGAGTTGCTTC. Xnr3 primers were AGGCAAAAGGTCTCCATCTG and TGCCCCATCCGATCTTCTG. noggin primers were AGTTGCAGATGTGGCTCT and AGTCCAAGAGTCTGAGCA. Amplification of all fragments was within the linear range of the PCR after 25 cycles except for *histone H4*, which was saturated after 24 cycles of PCR.

Luciferase Assays

The luciferase assays were performed as described previously (Watabe *et al.*, 1995).

RESULTS AND DISCUSSION

Organizer activity is positively regulated by activin/Vg1- and Wnt-type signals and antagonized by BMP signaling. In order to better understand the antagonistic effects mediated by BMPs, we examined the influence of BMPs on the expression of organizer-specific homeobox genes such as *Xsia*, *Xtwn*, and *Xgsc*. As *Xsia* and *Xtwn* expression is induced primarily in response to Wnt-type signals, the effects of BMP signaling on the expression of *Xtwn* and *Xsia* were examined. Furthermore, BMP antagonism of *Xgsc* expression was also studied to distinguish between BMP interference with either activin- or Wnt-mediated induction of the organizer since *Xgsc* induction requires synergistic input from both activin/Vg1- and Wnt-type signals.

In order to investigate the effects of BMP signaling upon the endogenous expression of these molecules, synthetic RNA encoding either a constitutively active or a dominant negative form of the *Xenopus* BMP type I receptor, CABR or DNBR, respectively (Suzuki *et al.*, 1994; Candia *et al.*, 1997), was injected into the dorsal marginal zone region of four-cell-stage embryos. Dorsal marginal explants were isolated from the prospective organizer region at blastula stage 9. At early gastrula stage 10.25 (when both *Xtwn* and *Xgsc* are normally expressed), RNA was harvested from explants derived either from uninjected embryos or from embryos injected with either CABR or DNBR mRNA. RNAs from these dorsal marginal zone explants were then subject to RT-PCR analysis. As shown in Fig. 1, injection of as much as 2 ng of either CABR or DNBR mRNA has no effect on *Xtwn* or *Xsia* expression relative to explants from uninjected embryos (lanes 2–6), whereas *Xgsc* expression is reduced in the presence of CABR (lanes 4 and 6). To demonstrate that BMP signaling is indeed active, the expression levels of the BMP-responsive gene *Xvent1* were analyzed by RT-PCR following injections of CABR and DNBR mRNA. As expected, *Xvent1* expression was enhanced in the presence of CABR (lanes 4 and 6). These results indicate that the expression levels of *Xtwn* and *Xsia* are not significantly affected by BMP-type signals while the expression level of *Xgsc* is regulated by BMP signals in a

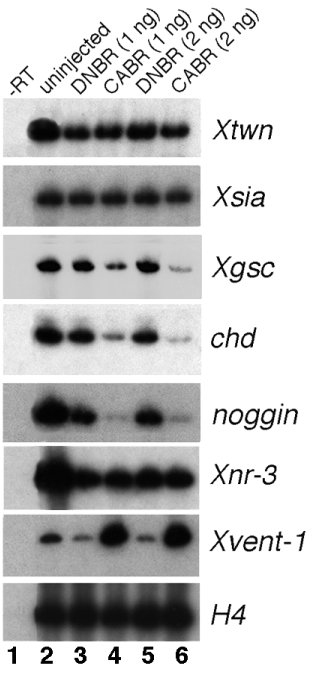

FIG. 1. RT-PCR analysis of *Xtwn, Xsia,* and *Xgsc* in explants derived from embryos injected with CABR or DNBR. The dorsal blastomeres of four-cell-stage embryos were injected with 1 or 2 ng of either constitutively active or dominant negative BMP receptor mRNA (CABR or DNBR, respectively). At gastrula stage 10.25, RNA from dorsal explants (dissected at blastula stages 8.5–9) was analyzed for *Xtwn, Xsia, Xgsc, chd, noggin, Xnr3, Xvent1,* and *histone H4* expression by RT-PCR. *histone h4* expression acts as a loading control. Although CABR and DNBR signaling affects *Xgsc, chd,* and *noggin* expression levels, the levels of *Xtwn* and *Xsia* remain unchanged by BMP signaling. These experiments were performed on three separate occasions with similar results obtained each time. One representative experiment is shown.

negative manner. Interestingly, we find that the expression of chordin and noggin is also significantly reduced in the presence of BMP signaling, while the expression of Xnr3 is relatively unaffected.

To confirm that BMP signaling does not significantly affect *Xtwn* expression, *Xtwn* and *Xgsc* reporter constructs were injected into the dorsal equatorial (C1), the ventral equatorial (C4), or the ventral animal (A4) blastomeres of 32-cell stage embryos. Embryos were harvested at gastrula stage 10.25 and monitored for luciferase activity. The *Xtwn* construct used, −357 *Xtwn*/Luc, contains all three LEF consensus binding sites which are required to mediate Wnt induction of *Xtwn* (Laurent *et al.*, 1997), while −226 *Xgsc*/Luc possesses both the DE and the PE, which respond to activin and Wnt signals, respectively (Watabe *et al.*, 1995). Two additional *Xgsc* promoter luciferase constructs, DE(6X)*Xgsc*/Luc, which comprises six copies of the DE, and −155 *Xgsc*/Luc, a construct which contains only the proximal element, were also injected to investigate the means by which BMP signaling exerts its effects on *Xgsc* expression. It has previously been shown that BMP signaling can affect DE-mediated induction of *Xgsc* (Candia *et al.*, 1997); however, the effects of BMP signaling on Wnt-mediated induction of *Xgsc* via the PE have not been analyzed. As shown in Fig. 2a, the presence of BMP signaling can reduce the endogenous induction of −226 *Xgsc*/Luc by 200-fold. In addition, as has been shown previously, BMP signaling can also significantly repress endogenous induction of the DE multimer-luciferase construct, DE(6X)*Xgsc*/Luc (Fig. 2a). Interestingly, BMP signaling also appears to have some repressive effects on PE-mediated induction of *Xgsc* as evidenced by the 5-fold level of repression of −155 *Xgsc*/Luc, although how this repression is orchestrated is still not clear. However, analysis using a reporter gene containing a multimerized PE(PE(5X)gsc/Luc) demonstrates that microinjection of dominant-negative Smad4 (DN-Smad4) can effectively block the induction of the reporter gene (Fig. 2b), suggesting the involvement of Smads in PE-mediated transcriptional regulation. Unlike the *Xgsc* luciferase constructs, expression of −357 *Xtwn*/Luc is not significantly affected by BMP signaling since the levels of induction in both the absence and the presence of BMP signaling have very similar profiles (Fig. 2a).

To further demonstrate that BMP signaling does not significantly affect *Xtwn*'s expression, −357*Xtwn*/Luc was injected either alone or with different concentrations of synthetic mRNA into the animal pole region of four-cell-stage embryos. Animal pole explants were collected from injected embryos at blastula stage 9 and allowed to develop in isolation until gastrula stage 10.25 at which point they were harvested and assayed for luciferase activity. As expected, injection of 30 and 100 pg of *XWnt8* mRNA could induce −357 *Xtwn*/Luc to activity levels of 13- and 27-fold, respectively, over injection of reporter gene alone (Fig. 2c). To determine whether BMP signaling can exert an effect on Wnt-mediated induction of *Xtwn,* CABR mRNA was coinjected with *XWnt8* mRNA into animal pole blastomeres. As shown in Fig. 2c, BMP signaling, at concentrations as high as 2 ng of CABR mRNA, did not significantly alter Wnt-mediated induction of *Xtwn* expression.

Data obtained by both RT-PCR and luciferase reporter gene analyses demonstrate that BMP signaling does not affect *Xtwn* or *Xsia* expression but can reduce *Xgsc* expression. According to these observations, we predict that overexpression of CABR should still be able to suppress the effect of *Xtwin/Xsia*-mediated secondary axis induction by blocking the expression of *Xgsc* and other downstream target genes. Indeed, the frequency of complete secondary axis (including head structures) formation induced by *Xtwin* overexpression is reduced drastically (92% (n = 12) to 0% (n = 25)) in the presence of CABR.

The suppression of *Xgsc* by BMP signaling appears to be mediated both through the DE and through the PE. The PE-mediated suppression of *Xgsc* by BMP signaling and DN-Smad4 is interesting in light of recent evidence suggesting that a complex composed of Smad2, Smad4, and

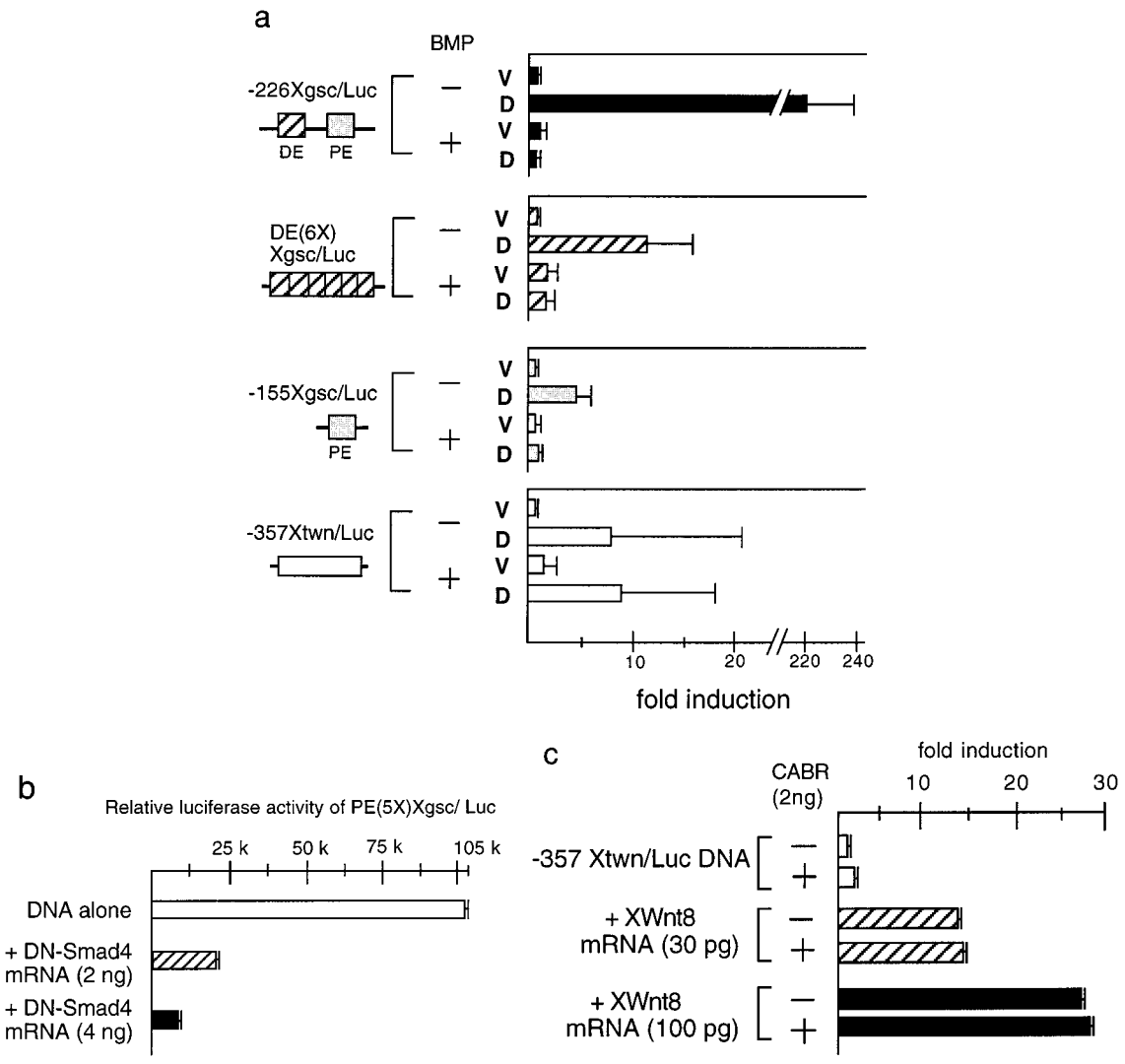

FIG. 2. Analysis of BMP signaling on *Xtwn* and *Xgsc* reporter genes. (a) 160 pg of −226 *Xgsc*/Luc, DE(6X)*Xgsc*/Luc, −155 *Xgsc*/Luc, or −357 *Xtwn*/Luc was injected into the dorsal (C1) (designated D), the ventral (C4) (designated V), or the animal (A4) (designated V) blastomere of the 32-cell-stage embryo. Luciferase constructs were injected into these blastomeres either in the absence (−) or in the presence (+) of 2 ng of synthetic mRNA encoding a constitutively active BMP receptor (CABR). The distal and the proximal elements of the *Xgsc* promoter are indicated by DE and PE, respectively. Experiments were performed a minimum of three times, each with the standard deviation indicated by error bars. The average levels of fold induction relative to background, A4 levels of activity are indicated. While BMP signaling can repress the induction of the *Xgsc* reporter genes −226 *Xgsc*/Luc, DE(6X)*Xgsc*/Luc, and −155 *Xgsc*/Luc, expression of −357 *Xtwn*/Luc is not significantly affected by active BMP signaling. (b) 160 pg of PE(5X)gsc/Luc was injected into the C1 cell with increasing concentrations of DN-Smad4 mRNA. Overexpression of dominant-negative Smad4 blocked the expression of PE(5X)gsc/Luc reporter gene in the C1 cell. (c) −357 *Xtwn*/Luc, a luciferase construct which is sufficient to mediate Wnt-type induction of *Xtwn*, was injected either alone or with synthetic RNA into the animal pole region of 4-cell-stage embryos. Animal pole explants were collected at blastula stage 9 and assayed for luciferase activity at gastrula stage 10.25. 30 and 100 pg of synthetic *XWnt8* mRNA is able to induce −357 *Xtwn*/Luc to activity levels of 13- and 27-fold, respectively. However, the presence of BMP signaling via co-injection of 2 ng of CABR synthetic mRNA does not alter Wnt-mediated *Xtwn* induction, indicating that BMP signaling is unable to significantly affect *Xtwn* expression. These experiments were performed on three separate occasions with similar results obtained each time. A single representative experiment is shown.

FAST2 binds to this region to mediate activin signaling (Labbe *et al.*, 1998). Perhaps in the presence of BMP signaling, induction of *Xgsc* via the PE is attenuated via Smad competition as has been suggested previously for the DE (Candia *et al.*, 1997). We propose a model whereby BMPs antagonize organizer activity by interfering with the induc-

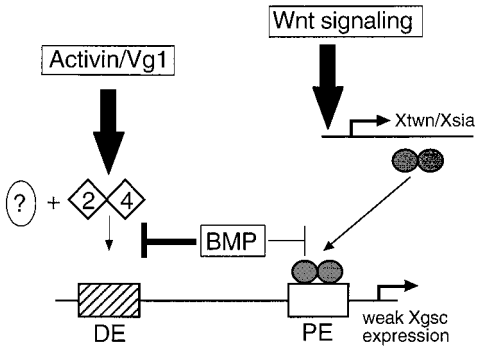

FIG. 3. Model of BMP antagonism of organizer-specific genes. *Xgsc* expression is mediated via the combined effects of two regions of its promoter, the distal element (DE) and the proximal element (PE). The DE responds directly to mesoderm-inducing signals such as activin, whereas the PE responds indirectly to Wnt signaling, i.e., following the induction of the *Xtwn/Xsia* homeobox genes. Activin stimulates the association of Smad2 and Smad4 (numbered diamonds) with an as yet unidentified factor (ovals containing question marks). *Xtwn/Xsia* expression is induced primarily by Wnt signaling. The homeodomain(s) of the *Xtwn* and/or *Xsia* proteins binds to the PE of the *Xgsc* promoter, as monomers, homodimers, or heterodimers. Hypothetical dimeric forms of *Xtwn/Xsia* (black circles) are shown here since the *Xtwn/Xsia* binding sites within the PE contain inverted repeats. In the presence of BMP signaling, induction of *Xgsc* is attenuated, possibly due to the intracellular competition for shared signaling components required by the DE and the PE.

tion of genes active in the organizer, such as *Xgsc*, but do not affect the expression of genes such as *Xtwn* and *Xsia* which are involved in initial organizer formation (Fig. 3). Thus ventralization produced by BMP signaling is clearly different from that of UV irradiation as it occurs at a later time than the activities initiated by the dorsal determinants. UV irradiation blocks the distribution of the dorsal determinants during cortical rotation, while BMP signaling inhibits the expression of genes involved in organizer activity.

In *Drosophila*, genetic analysis has revealed that BMP and Wnt signaling can interact to affect growth and patterning in the embryo. In the patterning of the *Drosophila* leg discs, it has been demonstrated that antagonism between Wingless (Wg; homolog of vertebrate Wnt) and Decapentaplegic (Dpp; homolog of vertebrate BMPs) signals establishes distinct regions of gene expression (Jiang and Struhl, 1996; Johnston and Schubiger, 1996; Theisen *et al.*, 1996). Furthermore, endoderm formation requires input from both Wg and Dpp signaling since the midgut enhancer of the *Ultrabithorax* gene possesses adjacent Wg- and Dpp-responsive elements which act coordinately to enhance *Ubx* expression (Riese *et al.*, 1997). While this genetic evidence shows that Wg and Dpp do interact, the molecular mechanism for this interaction is not clear. While Wg and Dpp could crosstalk intracellularly, it is equally possible that Wg indirectly regulates the expression of Dpp to activate or repress Dpp signaling and/or vice versa. In the case of *Xenopus* organizer formation, our analysis reveals that BMP signaling does not converge intracellularly with Wnt signaling at the level of the dorsal determinants and suggests that BMP and Wnt signaling function independently.

Recently, it has been shown that activin-like signaling via Smad2 enhances Wnt-mediated induction of *Xsia* (Crease *et al.*, 1998). However, at the present time, it is not clear as to how the activin/BVg1-mediated Smad2 pathway is involved in modulating the expression of *Xsia*. One interpretation might be that activation of the activin pathway indirectly participates in Wnt-mediated *Xsia* and *Xtwn* induction by triggering the activity of signaling components involved in their expression, rather than the activin pathway being directly involved in the induction of genes such as *Xtwn/Xsia*. Our results strongly suggest that the mechanism by which Smad2-mediated induction of *Xtwn* and *Xsia* occurs is distinct from that of *Xgsc*, since BMP signaling is unable to repress *Xtwn* and *Xsia* expression.

The observation that BMP signaling does not affect the gene expression of *Xsia* and *Xtwn* (genes that are turned on earlier than *Xgsc*), yet influences the gene expression of *Xgsc* and potential downstream target genes such as chordin and noggin, suggests that BMPs may exert their effects postzygotically during late blastula to early gastrula stages. Our previous work has also suggested that induction of the organizer requires two phases of regulation: an initiation establishment phase and a maintenance phase involving stable expression of organizer-specific genes (Artinger *et al.*, 1997). It may be that *Xtwn* and *Xsia* are involved in the initiation phase of organizer formation while the maintenance phase of organizer function (possibly mediated by continuous expression of *Xgsc*) is inhibited in the presence of BMP signaling. Further characterization of the regulation of the *Xtwn*, *Xsia*, and *Xgsc* promoters by Wnt, activin, and BMP signaling will be necessary to better understand the mechanisms of organizer formation.

ACKNOWLEDGMENTS

The authors thank Drs. Ira Blitz and Ted Brummel and reviewers for their reading of the manuscript and helpful comments and the members of the Cho lab for their help and assistance. This work was supported by an NIH grant to K.W.Y.C. (GM-54704).

REFERENCES

Artinger, M., Blitz, I. L., Inoue, K., Tran, U., and Cho, K. W. Y. (1997). Interaction of *goosecoid* and *brachyury* in *Xenopus* mesoderm patterning. *Mech. Dev.* **65,** 187–196.

Behrens, J., Vonkries, J. P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* **382,** 638–642.

Blitz, I. L., and Cho, K. W. (1995). Anterior neurectoderm is progressively induced during gastrulation: The role of the *Xenopus* homeobox gene orthodenticle. *Development* **121,** 993–1004.

Brannon, M., Gomperts, M., Sumoy, L., Moon, R. T., and Kimelman, D. (1997). A beta-catenin/XTcf-3 complex binds to the Siamois promoter to regulate dorsal axis specification in *Xenopus*. *Genes Dev.* **11,** 2359–2370.

Candia, A. F., Watabe, T., Hawley, S. H., Onichtchouk, D., Zhang, Y., Derynck, R., Niehrs, C., and Cho, K. W. (1997). Cellular interpretation of multiple TGF-beta signals: Intracellular antagonism between activin/BVg1 and BMP-2/4 signaling mediated by Smads. *Development* **124,** 4467–4480.

Cho, K. W., Morita, E. A., Wright, C. V., and De Robertis, E. M. (1991). Overexpression of a homeodomain protein confers axis-forming activity to uncommitted *Xenopus* embryonic cells. *Cell* **65,** 55–64.

Crease, D. J., Dyson, S., and Gurdon, J. B. (1998). Cooperation between the activin and Wnt pathways in the spatial control of organizer gene expression. *Proc. Natl. Acad. Sci. USA* **95,** 4398–4403.

Dale, L., Howes, G., Price, B. M., and Smith, J. C. (1992). Bone morphogenetic protein 4: A ventralizing factor in early *Xenopus* development. *Development* **115,** 573–585.

Fan, M. J., and Sokol, S. Y. (1997). A role for Siamois in Spemann organizer formation. *Development* **124,** 2581–2589.

Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rowning, B., and Stewart, R. (1989). Cortical rotation of the *Xenopus* egg: Consequences for the anteroposterior pattern of embryonic dorsal development. *Development* **107 Suppl.,** 37–51.

Hawley, S. H., Wunnenberg-Stapleton, K., Hashimoto, C., Laurent, M. N., Watabe, T., Blumberg, B. W., and Cho, K. W. (1995). Disruption of BMP signals in embryonic *Xenopus* ectoderm leads to direct neural induction. *Genes Dev.* **9,** 2923–2935.

Holowacz, T., and Elinson, R. P. (1993). Cortical cytoplasm, which induces dorsal axis formation in *Xenopus*, is inactivated by UV irradiation of the oocyte. *Development* **119,** 277–285.

Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G., and Kemler, R. (1996). Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. *Mech. Dev.* **59,** 3–10.

Jiang, J., and Struhl, G. (1996). Complementary and mutually exclusive activities of decapentaplegic and wingless organize axial patterning during *Drosophila* leg development. *Cell* **86,** 401–409.

Johnston, L. A., and Schubiger, G. (1996). Ectopic expression of wingless in imaginal discs interferes with decapentaplegic expression and alters cell determination. *Development* **122,** 3519–3529.

Jones, C. M., Dale, L., Hogan, B. L., Wright, C. V., and Smith, J. C. (1996). Bone morphogenetic protein-4 (BMP-4) acts during gastrula stages to cause ventralization of *Xenopus* embryos. *Development* **122,** 1545–1554.

Jones, C. M., Lyons, K. M., Lapan, P. M., Wright, C. V., and Hogan, B. L. (1992). DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in *Xenopus* mesoderm induction. *Development* **115,** 639–647.

Kageura, H. (1997). Activation of dorsal development by contact between the cortical dorsal determinant and the equatorial core cytoplasm in eggs of *Xenopus laevis*. *Development* **124,** 1543–1551.

Kessler, D. S. (1997). Siamois is required for formation of Spemann's organizer. *Proc. Natl. Acad. Sci. USA* **94,** 13017–13022.

Labbe, E., Silvestri, C., Hoodless, P., Wrana, J., and Attisano, L. (1998). Smad2 and Smad3 positively and negatively regulate TGFb-dependent transcription through the forkhead DNA-binding protein FAST2. *Mol. Cell* **2,** 109–120.

Laurent, M. N., Blitz, I. L., Hashimoto, C., Rothbacher, U., and Cho, K. W. (1997). The *Xenopus* homeobox gene twin mediates Wnt induction of goosecoid in establishment of Spemann's organizer. *Development* **124,** 4905–4916.

Lemaire, P., Garrett, N., and Gurdon, J. B. (1995). Expression cloning of Siamois, a *Xenopus* homeobox gene expressed in dorsal-vegetal cells of blastulae and able to induce a complete secondary axis. *Cell* **81,** 85–94.

Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O., and Clevers, H. (1996). XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos. *Cell* **86,** 391–399.

Riese, J., Yu, X., Munnerlyn, A., Eresh, S., Hsu, S. C., Grosschedl, R., and Bienz, M. (1997). LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. *Cell* **88,** 777–787.

Scharf, S. R., and Gerhart, J. C. (1983). Axis determination in eggs of *Xenopus laevis:* A critical period before first cleavage, identified by the common effects of cold, pressure and ultraviolet irradiation. *Dev. Biol.* **99,** 75–87.

Suzuki, A., Thies, R. S., Yamaji, N., Song, J. J., Wozney, J. M., Murakami, K., and Ueno, N. (1994). A truncated bone morphogenetic protein receptor affects dorsal–ventral patterning in the early *Xenopus* embryo [see comments]. *Proc. Natl. Acad. Sci. USA* **91,** 10255–10259.

Theisen, H., Haerry, T. E., O'Connor, M. B., and Marsh, J. L. (1996). Developmental territories created by mutual antagonism between Wingless and Decapentaplegic. *Development* **122,** 3939–3948.

Watabe, T., Kim, S., Candia, A., Rothbacher, U., Hashimoto, C., Inoue, K., and Cho, K. W. (1995). Molecular mechanisms of Spemann's organizer formation: Conserved growth factor synergy between *Xenopus* and mouse. *Genes Dev.* **9,** 3038–3050.

Received for publication August 20, 1998
Revised November 12, 1998
Accepted November 12, 1998